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Effects of Low-Energy Gallium-Aluminum-Arsenide Laser Irradiation on Cultured Fibroblasts and Keratinocytes

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Background and Objective: **To assess whether the gallium-aluminum-arsenide low energy laser will increase cell proliferation, cell attachment, or cell migration in cultured fibroblasts and keratinocyte models.**
Study Design/Materials and Methods: **Monolayer cultures of fibroblasts and keratinocytes were subjected to gallium-aluminum-arsenide laser irradiation at varying power densities for varying time intervals. Cell proliferation was assessed by absorbent spectrophotometry while cell adhesion was assessed by a microcolorimetric assay for cells attached to bovine dermis collagen. Cell migration was assessed through a filter utilizing high power microscopic fields.**
Results: **There were no differences in cell proliferation, adhesion, or migration in either the fibroblast or keratinocyte culture treated with the gallium-aluminum-arsenide laser at any power density or time compared with nontreated controls.**
Conclusion: **The gallium-aluminum arsenide laser, when utilized at powers of 5–100 milliwatts and times of between 10–120 seconds has no biostimulatory effect on fibroblasts or keratinocyte cultures as assessed by cell proliferation, adhesion, or migration. Lasers Surg. Med. 20:426–432, 1997.** © 1997 Wiley-Liss, Inc.

Key words: soft lasers; Ga-Al-As laser; laser biostimulation

INTRODUCTION

The therapeutic value of low-energy lasers (also known as soft lasers) is controversial. Their use was first advocated in the 1960s by the late Dr. Andre Mester in Hungary and much of the early work was carried out in Eastern Europe and the Soviet Union [1–3]. Anecdotal reports have appeared on the use of low energy lasers in many different medical situations including wound healing [4,5], healing of apthous ulcers [6], inhibition of plaque formation on teeth [7], enhanced healing of dental extraction sockets [8], recovery from nerve injuries [9], and its use in painful temporomandibular joint disorders and other fibromyalgic conditions [10,11]. Reports have even appeared of improvements in areas distant from those irradiated [12]. However, double-blind and controlled studies have generally failed to show beneficial effects from these lasers [13,14] and the FDA withdrew approval for their therapeutic use in the USA in 1983 [15]. More recently in vitro studies have shown effects on tissue cultures, including increased production of pro-collagen, col-

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lagen, and increased proliferation of fibroblasts exposed to low energy laser irradiation [5,16].

Low energy lasers transmit energy at low enough levels in the tissues that any temperature elevation is less than 0.1°C [17]. This is in contrast to high energy lasers such as the carbon dioxide laser and neodymium-YAG laser which can raise tissue temperatures high enough to cut and vaporize them [18]. The lasers normally utilized for low energy studies are either the helium neon (He-Ne) laser at a wavelength of 632 nm which transmits a red light or the gallium-aluminum arsenide (Ga-Al-As) laser with a wavelength of 830 nm which is in the infrared portion of the spectrum, and therefore invisible to the human eye. Most in vitro studies have been carried out on the He-Ne laser though clinically, in Europe and Asia, the Ga-Al-As laser appears to have more popularity at the present time [13,14].

This study represents an in vitro study with a Ga-Al-As laser with a power output of between 5 and 100 mw and assesses its effects on both fibroblast and keratinocyte cell cultures.

MATERIALS AND METHODS

Cell Culture

Two cell lines were used in this study, consisting of human lung fibroblasts (WI38VA13) and human foreskin keratinocytes (HFK). These cells were subcultured in Dulbecco's modified Eagle's medium (DME) containing 100 IU/ml penicillin, 100 μg/ml streptomycin, and 2 ml glutamine, supplemented with 10% fetal calf serum in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. The medium was changed at 2-day intervals. Prior to laser treatment, the cells were removed by trypsinization and counted by hemocytometer. Fibroblasts and keratinocytes (1×10^4, 2×10^4 cells) in 0.2 ml of medium, were plated on 96-well ELISA-type tissue culture plates, the diameter of each well being 0.635 cm. The cells were allowed to attach for 24 hours prior to treatment. Immediately prior to laser treatment, the medium was removed and replaced with 20 μl of phosphate-buffered saline (PBS). The cells were then individually perpendicularly irradiated by the laser which was applied to the cell culture at 1.1 cm irradiation distance. The distance represents the depth of the wells, i.e., the laser touched the top surface of the well to irradiate the cells coated on the base of the well. Control cultures were handled in an identical fashion but without laser irradiation. Following irradiation, the cells were incubated for either 24 or 48 hours.

Laser

The Ga-Al-As laser (Diolase 100, Berkeley, CA) was used in this study. This laser is continuously adjustable from 5–100 mW, with a wavelength of 830 nm. The laser diode has an ellipsoid beam spot. The greatest diameter of the spot at the study distance of 1.1 cm from the laser probe was 0.425 cm. The time of exposure was between 10 and 120 seconds with a power of between 5 and 100 mw. This translates to a power density of from 0.12 to 4.24 J/cm^2 (from a low of 5 mw for 10 secs to a high of 100 mw for 120 secs).

For each of the experiments described below, nine wells of a 96-well plate were used for each individual procedure. The wells were irradiated at powers of 5 mw, 10 mw, 20 mw, 30 mw, 45 mw, 60 mw, and 100 mw. Times used were 10 sec, 20 sec, 30 sec, 45 sec, 60 sec, and 120 sec to give a total of 42 different time and power combinations. Each assay was carried out in duplicate or triplicate and were incubated for either 24 hours or 48 hours. This gives a total combination of power, time of irradiation, and time of incubation of 84 combinations. Non-irradiated controls were run for each procedure as described above.

Measurement of Cell Numbers

The number of cells within the wells of the 96-well tissue culture plates were quantitated by a sensitive and simple microcolorimetric assay. Briefly, 24 and 48 hours after laser irradiation, the cells were washed twice with PBS and fixed with 1% formaldehyde (50 μl/well) for 2–3 minutes. Then, the cells were stained with 2% crystal violet (50 μl/well) for 10 minutes. After washing the plates with distilled water, 1% sodium dodocil sulfate (SDS) (50 μl/well) was added into each well to dissolve the cells. The plates were directly scanned in a plate spectrophotometer (Bio-Tek Instruments, Winooski, VT) at 562 nm and the resultant absorbance taken as proportional to the number of cells present. The assay for each laser irradiation dose was carried out in triplicate (nine wells/group) and the data are expressed as the mean plus standard deviation.

Cell Attachment

The effect of laser irradiation on the attachment of cells to tissue culture plates coated with bovine dermis collagen I (Celtrix, Palo Alto, CA) was monitored in a second series of experiments.

Each well of a 96-well tissue culture plate was precoated with 50 μl of collagen I (0.6 μg/ml) at 37°C for 1 hour and then washed three times with PBS, blocked with 0.1% bovine serum albumin (BSA) in the DME at 37°C for 1 hour. Seventy percent confluent cells were detached from dishes with 0.25% trypsin and resuspended in PBS at a final concentration of 1×10^4 cells/ml. Twenty microliters of cell suspension were placed in each well of a 96-well plate. The cells were then immediately irradiated with the laser and the plate was then placed into a CO_2 incubator. The cells were allowed to attach for 30 minutes at 37°C, and attached cells were quantified by a microcolorimetric assay utilizing crystal violet as described above. The total cells bound to the control collagen I substrate are indicated as 100% and the experimental results presented as a percentage above or below the 100% for the control.

Cell Migration Assay

Fibroblast migration was assayed in a modified Boyden chamber (Neuroprobe, Bethesda, MD) with 8-mm polyvinyl pyrolidone-free poly--carbonate filters (Nucleopore, Pleasanton, CA). The undersides of the filters were precoated with 5 μg/ml of bovine dermis collagen I. The lower well of the chamber was filled with 0.1% BSA (26 μl/well). The fibroblasts in culture were then treated with the laser at the range of irradiation doses described and were then resuspended to a final concentration of 4×10^4 cells/ml in serum-free DME supplemented with 0.1% BSA. A 50 μl aliquot of cell suspension was added to the upper well of the chamber and incubated for 4 hours at 37°C, after which migration to the underside of the precoated filter was stained with crystal violet as previously described and measured. For each chamber, the number of migrated cells in four randomly chosen high power fields (magnification, × 400) was counted.

This experiment was only performed on the fibroblasts while the others were performed on both the fibroblasts and keratinocytes.

RESULTS

The cell quantitation assay demonstrated that Ga-Al-As laser irradiated cultures (both fibroblasts and keratinocytes) failed to produce an increase in the number of cells in comparison with their respective non-irradiated controls (Fig. 1). Although the total irradiation doses used in our study ranged from 0.12–4.24 J/cm^2, which is well within the recommended limits, we could not observe any significant effect of Ga-Al-As laser on cell growth. We also investigated the effect of laser irradiation on cell attachment to collagen I substrate and again no significant change in cell attachment was observed in either cell line following laser irradiation (Fig. 2). The cell migration study also did not indicate that laser irradiation could increase the fibroblast migratory response to collagen I (Fig. 3).

DISCUSSION

Low level energy lasers have been proposed to have effects on tissue metabolism that have been broadly termed "laser biostimulation." One theory of the action of low level energy is that the laser is capable of influencing photoreceptors in the cells, thereby producing a stimulation of cell proliferation [9,19–21]. Although some experimental studies claim to provide evidence in relation to the effect of low energy He-Ne laser irradiation on fibroblast proliferation [4,5,10], whether or not the Ga-Al-As laser (wavelength: 830 nm) has any similar stimulative effect on fibroblasts or skin keratinocytes in vitro remains unclear [5].

Haas et al. [22] showed that the He-Ne laser increased keratocyte motility but not proliferation, while Abergel et al. [23] utilized both the He-Ne and Ga-Al-As lasers on fibroblast cultures and showed that both increased procollagen production but not cellular proliferation, and the cell line behaved differently with the different lasers. The present study did not confirm that exposure to the Ga-Al-As laser would have any stimulatory effects on either cultured fibroblasts or keratinocytes. Previous investigators have emphasized the importance of choosing the correct laser energy level [20]. Though the recommended energy density for obtaining optimal "biostimulation" varies in the published literature [24,25], the total laser doses utilized in the present study ranged from 0.12–4.24 J/cm^2 depending on the power and exposure time, and were within the ranges suggested in previous studies. As with most low energy laser studies, the fibroblasts and keratinocytes were cultured separately. Explant assays as described by Halprin et al. [26] may give different results and simulate an in vivo model since it would allow interaction between the different cells. However, one study with the HeNe laser on the human skin explant model could detect no biostimulatory effect [27].

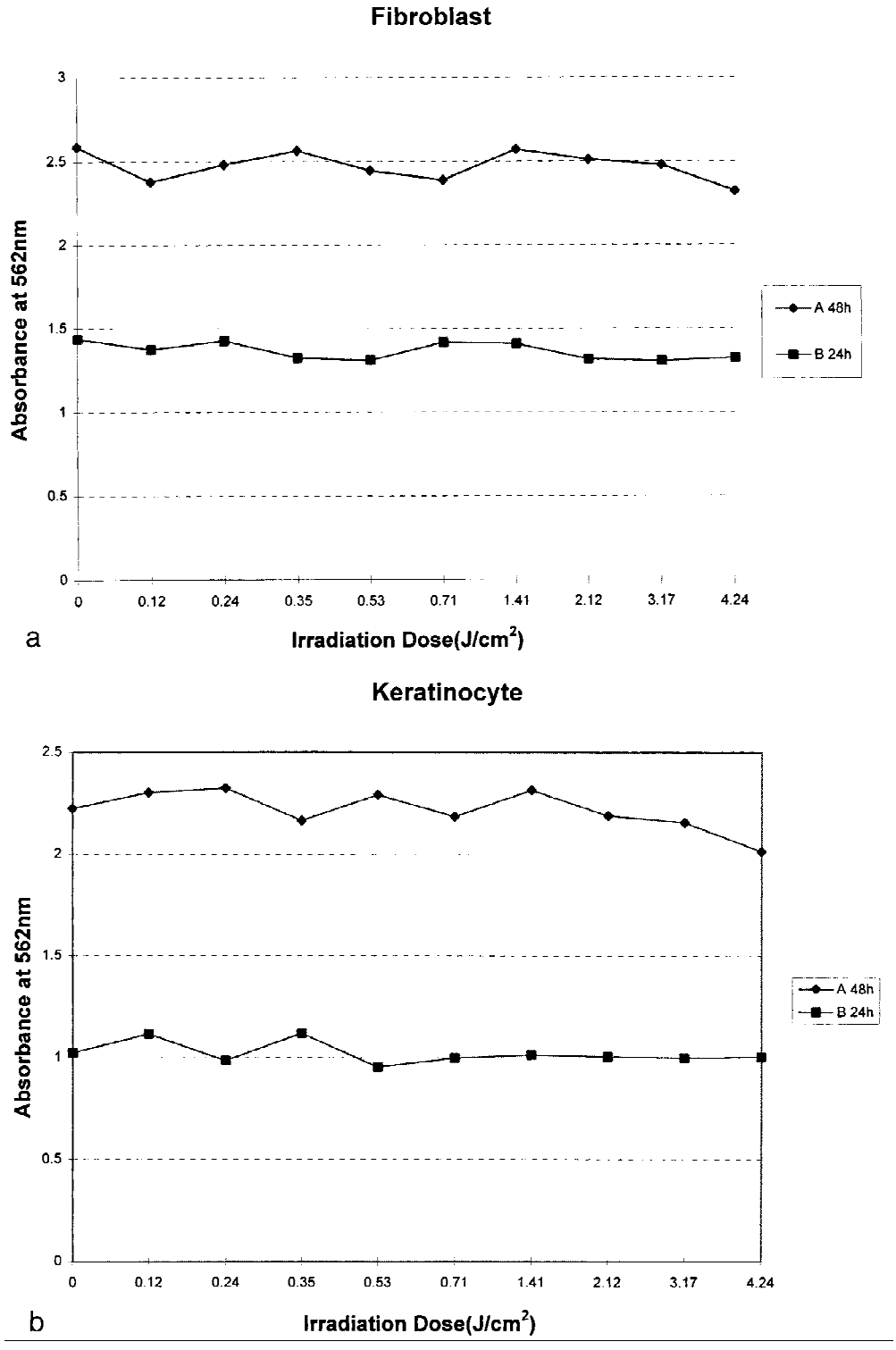

Fig. 1. Effect of Ga-Al-As laser irradiation on fibroblast (**a**) and keratinocyte (**b**) growth. Cell concentration per well was 1×10^4 cells. The number of cells was quantitated by a plate spectrophotometer at 562 nm. Each symbol and point indicates the mean and SD in a triplicate assay. Laser irradiation failed to produce an increase in the number of cells at a dose range between 0.12 J/cm^2 and 4.24 J/cm^2. A: 48 hours after irradiation; B: 24 hours after irradiation.

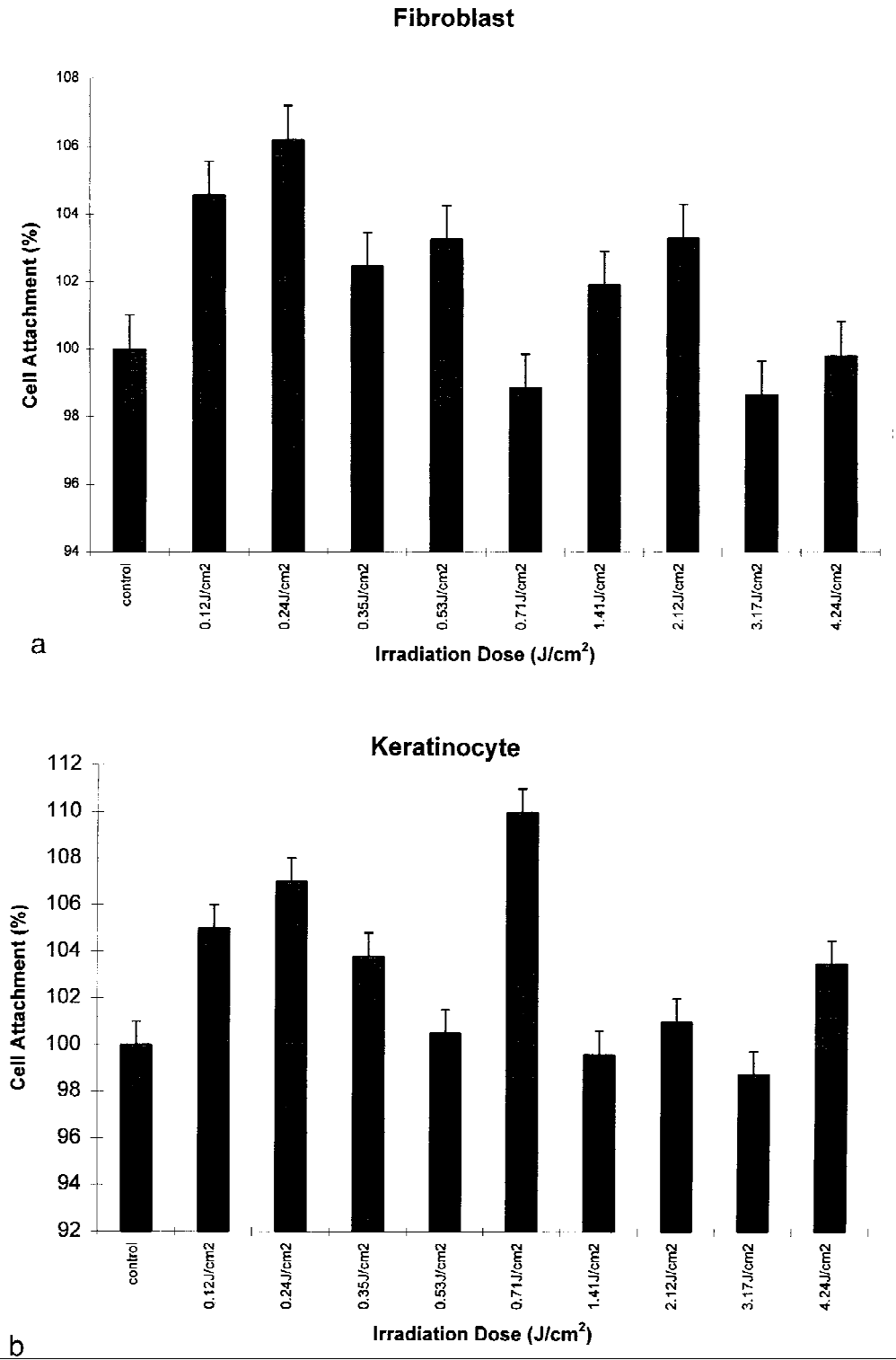

Fig. 2. Effect of Ga-Al-As laser irradiation on fibroblast (**a**) and keratinocyte (**b**) attachment to collagen I. Following irradiation, cells (2×10^4/well) were allowed to attach to plastic wells coated with collagen I (0.6 μg/ml). Data points and bars represent the mean and SD of triplicate wells. No significant difference exists between the control group and other irradiation groups.

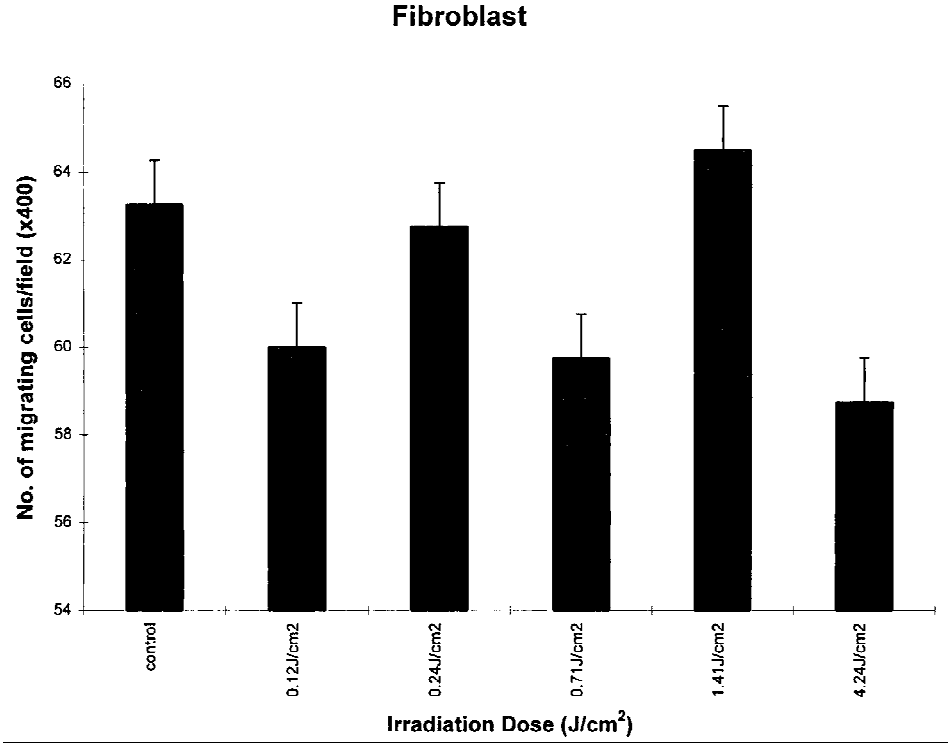

Fig. 3. Effect of Ga-Al-As laser irradiation on migration of fibroblasts. Fibroblasts were pretreated with laser at different irradiation doses. Migration was measured using the modified Boyden chamber assay as described in Materials and Methods. Each bar represents the mean and SD of four high power fields (× 400).

It would therefore appear that using parameters and doses recommended elsewhere we could detect no "biostimulating" activity of the gallium-aluminum-arsenide laser on fibroblasts or keratinocytes as assessed by cell proliferation, attachment, or migration.

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